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07541800 BIOSIS NO.: 000091093878

DETECTION OF CELL-CAM 105 IN THE PERICANALICULAR DOMAIN OF THE RAT HEPATOCYTE PLASMA MEMBRANE

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JOURNAL: HEPATOLOGY 13 (1). 1991. 47-56. 1991
FULL JOURNAL NAME: HEPATOLOGY (Baltimore)
CODEN: HPTLD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Cell-CAM 105 has been identified as a cell adhesion molecule based on the ability of anti-cell-CAM 105 **monospecific Fab** fragments to inhibit the reaggregation of rat hepatocytes. Because of its adhesive properties, it was expected that cell-CAM 105 would be present on the lateral cell surface where adhesive interactions predominate. Paradoxically, however, immunofluorescence analysis of frozen sections of rat liver using specific monoclonal antibodies indicated that cell-CAM 105 was present exclusively in the bile canalicular domain of the rat hepatocyte where there is no intercellular adhesion. To more precisely define the in situ localization of cell-CAM 105, immunoperoxidase labeling and electron microscopy were used to examine intact and mechanically dissociated liver tissue. Results showed that when accessibility was provided by mechanical dissociation of perfusion fixed liver tissue, cell-CAM 105 could be detected in the pericanalicular region of lateral membranes. In contrast, when hepatocytes were labeled after incubation in vitro under conditions used during adhesion assays to induce reaggregation, cell-CAM 105 rapidly redistributed to all areas of the plasma membrane. Immunofluorescence analysis of primary hepatocyte cultures further revealed that cell-CAM 105 and two other bile canalicular proteins relocalized to discrete domains reminiscent of bile canaliculi, whereas cell-CAM 105 was also present in areas of intercellular contact. Serial section electron microscopy analysis of well-defined, cross-sectional profiles of bile canaliculi suggested the presence of cell-CAM 105-positive membrane folds that extended along the length of the bile canalicular border. In sections from livers in which calcium-dependent adhesive contacts had been disrupted by treatment with ethylenediamine tetraacetate, intact bile canaliculi were found that remained attached only by these border folds. The implications of these results are discussed with regard to a possible role for cell-CAM 105 in bile canalicular formation.

6/9/2 (Item 2 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

06094462 BIOSIS NO.: 000085057611
C4-BINDING PROTEIN PREVENTS SPONTANEOUS CLEAVAGE OF C3 IN SERA OF PATIENTS WITH HEREDITARY ANGIOEDEMA
AUTHOR: GRONSKI P; BODENBENDER L; KANZY E-J; SEILER F R
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JOURNAL: COMPLEMENT 5 (1). 1988. 1-12. 1988
FULL JOURNAL NAME: Complement
CODEN: CMPLD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: We have studied the effects of polyclonal **monospecific Fab** ' preparations against C.hivin./r, C.hivin./s, C.hivin./INH, C4, C4bp, and fragmented Bb of factor B on complement activation in NHS and HAES. Furthermore, we have investigated complement activation in these sera after addition of purified C.hivin./s and purified C4bp. Blocking C.hivin./INH induced a spontaneous activation of the classical pathway in NHS and to a lesser extent in HAES. Addition of p-C.hivin./s resulted in a strong C3 conversion in NHS, but not in HAES. However, after the blocking of C4bp in HAES, addition of p-C.hivin.s produced a total C3 consumption. The ratio of the protein concentration of C4bp to hemolytically active C4 was eight times higher in HAES than in NHS. This increased ratio may account for the resistance of HAES to the C.hivin.s induced C3 cleavage in our in vitro system and the stability of C3 in HAE despite C4 and C2 consumption in vivo.
6/9/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05271865 BIOSIS NO.: 000082112490
EVOLUTION AND FUNCTION OF STRUCTURALLY DIVERSE SUBUNITS IN THE RESPIRATORY PROTEIN HEMOCYANIN FROM ARTHROPODS
AUTHOR: MARKI J
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JOURNAL: BIOL BULL (WOODS HOLE) 171 (1). 1986. 90-115. 1986
FULL JOURNAL NAME: Biological Bulletin (Woods Hole)
CODEN: BIBUB
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Native aggregation level and subunit composition of the hemocyanins from 86 adult chelicerates and crustaceans, and from the larval stages of 2 crabs, were analyzed by means of electron microscopy, polyacrylamide electrophoresis, immuno blotting, and crossed immunoelectrophoresis, supported by a variety of preparative separation techniques. The up to eight immunologically discernible subunit types were interspecifically correlated, classified, and evolution lines derived. Phylogenetic consequences are discussed, and are particularly aggravating in spiders. A single subunit suffices for the formation of hexamers (1 .times. 6). In the architecture of higher-ordered hemocyanins, the various subunits act as building-blocks of distinct specification. This was studied in 2 .times. 6 molecules from a spider and several crustaceans, and in 4 .times. 6 hemocyanin from a tarantula. The various subunits are present in constant proportions. The total set is required to reorganize the original aggregate from subunit mixtures. Stable oligomeric segments of native hemocyanin particles revealed the gross distribution of the diverse subunits. Immuno electron microscopy of the native hemocyanins decorated with **monospecific Fab** fragments showed the exact topographic position of each subunit type, and detailed models of the quaternary structure could be derived. The oxygen binding function of 4 .times. 6 hemocyanin from the tarantula Eurypelma californicum is excessively modulated by subunit interaction phenomena. We measured native, reassembled, and mercury-blocked 4 .times. 6-mers, oligomeric segments, single subunits, and reassembled 4 .times. 6-mers

with one subunit type chemically modified. The spatial range of allosteric interaction, and specific contributions of the diverse subunits are outlined.

6/9/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04362373 BIOSIS NO.: 000078091918
ALTERED SURFACE DISTRIBUTION OF BOTH COMPLEMENT C-3B RECEPTORS AND FC RECEPTORS ON NEUTROPHILS INDUCED BY ANTI C-3B RECEPTOR OR AGGREGATED IMMUNO GLOBULIN G
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JOURNAL: J IMMUNOL 132 (6). 1984. 3028-3033. 1984
FULL JOURNAL NAME: Journal of Immunology
CODEN: JOIMA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Human neutrophils to which **monospecific Fab** ' or F(ab')2 anti-C3b [complement component 3b] receptor was bound at 0.degree. C were incubated for timed intervals at temperatures ranging from 0.degree.-37.degree. C, after which the cells were labeled with TRITC[tetramethylrhodamine isothiocyanate]-conjugated 2nd antibody. Neutrophils bearing Fab' anti-C3b receptor and incubated for up to 30 min at 37.degree. C, and cells bearing F(ab')2 anti-C3b receptor and incubated at 0.degree. C, exhibited diffusely distributed punctate clusters of receptors. Neutrophils bearing the bivalent anti-receptor and incubated at 30.degree. C or 37.degree. C for 5 min had redistributed C3b receptors into caps and patches that were associated with subplasmalemmal accumulations of myosin. The redistribution of cross-linked C3b receptors was inhibited by pretreatment of the neutrophils with either cytochalasin D or chlorpromazine. On .apprx. 1/2 of the cells demonstrating capped C3b receptors there was a corresponding redistribution of Fc receptors, as demonstrated by subsequent binding of FITC[fluorescein isothiocyanate]-aggregated IgG (FITC agg-IgG). Capping of C3b receptors did not alter the diffuse distribution of HLA-A on these cells. Cross-linking of Fc receptors on neutrophils by FITC agg-IgG also induced temperature-dependent capping of these receptors that was inhibited by cytochalasin D and chlorpromazine. In .apprx. 1/2 of the cells demonstrating capped Fc receptors, subsequent labeling of C3b receptors revealed a similar redistribution of these receptors. The neutrophil responds to cross-linking of either C3b receptors or Fc receptors by a cytoskeletal-dependent rearrangement of both receptors that causes their overlapping topographic distribution, demonstrating a form of cooperative interaction between these 2 types of receptors that are involved in the phagocytic reactions of these cells.

6/9/11 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

02661681 EMBASE No: 1984130640
Altered surface distribution of both C3b receptors and Fc receptors on neutrophils induced by anti-C3b receptor or aggregated IgG
Jack R.M.; Fearon D.T.
Department of Medicine, Harvard Medical School, Boston, MA 02115 United States
Journal of Immunology (J. IMMUNOL.) (United States) 1984, 132/6 (3028-3033)
CODEN: JOIMA
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

Human neutrophils to which **monospecific Fab** ' or F(ab')2 anti-C3b receptor had been bound at 0degreeC were incubated for timed intervals at temperatures ranging from 0degreeC to 37degree C, after which the cells were labeled with TRITC-conjugated second antibody. Neutrophils bearing Fab' anti-C3b receptor and incubated for up to 30 min at 37degreeC, and

cells bearing F(ab')inf 2 anti-C3b receptor and incubated at 0degreeC, exhibited diffusely distributed punctate clusters of receptors. Neutrophils bearing the bivalent anti-receptor and incubated at 30degreeC or 37degreeC for 5 min had redistributed C3b receptors into caps and patches that were associated with subplasmalemmal accumulations of myosin. The redistribution of cross-linked C3b receptors was inhibited by pretreatment of the neutrophils with either cytochalasin D or chlorpromazine. On approximately one-half of the cells demonstrating capped C3b receptors there was a corresponding redistribution of Fc receptors, as demonstrated by subsequent binding of FITC-aggregated IgG (FITC agg-IgG). In contrast, capping of C3b receptors did not alter the diffuse distribution of HLA-A on these cells. Cross-linking of Fc receptors on neutrophils by FITC agg-IgG also induced temperature-dependent capping of these receptors that was inhibited by cytochalasin D and chlorpromazine. In approximately one-half of the cells demonstrating capped Fc receptors, subsequent labeling of C3b receptors revealed a similar redistribution of these receptors. Thus, the neutrophil responds to cross-linking of either C3b receptors or Fc receptors by a cytoskeletal-dependent rearrangement of both receptors that causes their overlapping topographic distribution, demonstrating a form of cooperative interaction between these two types of receptors that are involved in the phagocytic reactions of these cells.

01312683 SUPPLIER NUMBER: 11001717 (THIS IS THE FULL TEXT)
Monoclonal antibodies in diagnosis and therapy. (review article)
Waldmann, Thomas A.
Science, v252, n5013, p1657(6)
June 21,
1991
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Academic
WORD COUNT: 5796 LINE COUNT: 00561

ABSTRACT: A review is presented of the ways monoclonal antibodies are prepared for clinical use and of their effectiveness in diagnosing and treating disease. A monoclonal antibody is a protein produced by a cell of the immune system or by a genetically altered bacterium in response to a particular antigen (another protein). As such, it binds specifically to only that antigen and can, in theory at least, inactivate or enhance its activity. Examples of the conditions in which monoclonal antibodies have been used include autoimmune diseases, graft versus host disease, graft rejection, and certain viral infections. The targets on cell surfaces that serve as antigens are outlined; the CD3 antigen on T cells (the main cell type of the cellular immune system) is the target against which the monoclonal antibody OKT3 is directed. This is the monoclonal antibody in widest clinical use, and OKT3 is currently licensed for treating acute kidney graft rejection. Genetically engineered monoclonal antibodies combine regions of the mouse antibody with regions of the human antibody to maximize effectiveness. Such combinations have been tested against the antigens present on tumors. Problems in developing these agents are briefly discussed. Researchers are trying to produce human monoclonal antibodies by fusing mouse cells and human cells, or by DNA polymerase chain reaction technology (which allows copying antibody-producing genes). However, these monoclonal antibodies have not yet been tested in clinical trials. Antibodies have also been used to carry toxins or cell-killing (cytotoxic) drugs to specific targets on ceratin cells, such as tumor cells. A ''label'' that can be attached to antibodies is radioactivity (in the form of a radionuclide); then, when the antibody binds to its antigen located on, for instance, a tumor cell, it releases local radioactivity that kills the cell. Future clinical uses of monoclonal antibodies are described. (Consumer Summary produced by Reliance Medical Information, Inc.)

TEXT:

THE DEVELOPMENT OF MONOCLONAL ANTIBODY TECHNOLOGY by Kohler and Milstein [1] provided an enormous opportunity for examination of a range of previously elusive issues. For example, monoclonal antibodies are being

used in radioimmunoassays, enzyme-linked immunosorbent assays, immunocytopathology, and flow cytometry for in vitro diagnosis, and in vivo for diagnosis and immunotherapy of human disease. However, in the area of immunotherapy, monoclonal antibodies are just beginning to fulfill the promise inherent in their great specificity for recognizing and selectively binding to antigens on cells.

Monoclonal antibodies have largely been applied clinically to the diagnosis and therapy of cancer and the modulation of the immune response to produce immunosuppression for treatment of autoimmune and graft versus host diseases (GVHD) and for prevention of allograft rejection. Human monoclonal antibodies have also been applied clinically against cytomegalovirus, Varicella zoster virus, and the various specific serotypes of Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae. For example, in a multicenter clinical trial involving 200 patients with Gram-negative bacteremia, mortality was reduced in patients who received an immunoglobulin M (IgM) monoclonal antibody (HA-1A) that binds specifically to the lipid A domain of endotoxin [2]. In other studies, monoclonal antibodies that are specific for leukocyte adhesion molecules, such as lymphocyte functional antigen-1 (LFA-1) or intercellular adhesion molecule-1 (ICAM-1), the adhesion partner of LFA-1, have been used to inhibit accumulation of neutrophils and thereby reduce tissue damage in animal models of bacterial meningitis, hemorrhagic shock, and myocardial reprofusion injury [3].

The use of monoclonal antibodies has also been proposed for therapy of myocardial infarction [4], for reversal of drug toxicity (digitalis intoxication), and for fertility control. Despite this wide-ranging interest, the "magic bullet" of antibody therapy that has been the dream of immunotherapists since the time of Paul Ehrlich has proved to be elusive [5]. Only one monoclonal antibody, OKT3, has been licensed for clinical use. Furthermore, the initial use of unmodified murine monoclonal antibodies in human patients with cancer was disappointing, with only 23 partial and 3 complete remissions reported among the initial 185 patients included in 25 clinical trials [6]. A number of factors explain the low therapeutic efficacy observed. Unmodified murine monoclonal antibodies are immunogenic and elicit a human immune response to the murine antibodies. Moreover, most mouse monoclonal antibodies are not cytocidal against neoplastic cells in humans because the antibodies do not participate in human complement or cell-mediated cytotoxicity. In most cases, the antibodies were not directed against a vital cell-surface structure such as a receptor for a growth factor required for tumor cell proliferation. In an attempt to circumvent these problems, researchers have developed human and humanized antibodies, prepared by genetic engineering, that are less immunogenic than murine antibodies. Cytotoxic action has been augmented by arming the antibodies with toxins or radionuclides. Finally, cell surface antigenic targets have been defined for effective monoclonal antibody action.

In this article I summarize information on in vivo use of monoclonal antibodies for diagnosis and therapy, with special reference to the most seminal discoveries and recent advances in (i) definition of cell-surface structures on abnormal cells as targets for effective monoclonal antibody action, (ii) development of genetic engineering approaches for creating more effective agents, and (iii) development of techniques for arming monoclonal antibodies with radionuclides or toxins and thereby increasing effector function. Comprehensive recent reviews provide more detail [7-9].

Cell Surface Antigenic Targets

The ideal immunosuppressive monoclonal antibody would be one that abrogates responses to a defined antigen and preserves responses to all others. However, the monoclonal antibody that is most widely used clinically (OKT3) is directed against the CD3 (cluster of differentiation) antigen of the T cell receptor complex that is expressed on virtually all circulating T cells. The Food and Drug Administration licensed the use of OKT3 for the treatment of acute renal allograft rejection on the basis of randomized clinical trials showing the superiority of this treatment (93% reversal of acute rejection episodes) to more conventional, broad-spectrum immuno-suppressive agents (75% reversal) [10]. There are, however, toxicities associated with the use of OKT3. Antibodies to CD3 initiate in vivo T cell activation that is accompanied by release of tumor necrosis factor, interferon [gamma], and, in some cases, interleukin-2 (IL-2), which leads to an acute clinical syndrome that involves high fever, vomiting,

diarrhea, and occasionally respiratory distress. Furthermore, therapy with antibody to CD3 leads to broad immunosuppression that is associated with an increased incidence of infections and B cell neoplasms.

Monoclonal antibodies have been used to target the polymorphic [alpha] and [beta] subunits of the human T cell antigen receptor (TCR). In pilot studies, one monoclonal antibody (BMA 031) specific for the constant region of the TCR was used successfully in treatment of acute organ allograft rejection and GVHD [11]. Monoclonal antibodies that specifically identify the hypervariable antigen-binding region of the TCR represent more antigen-specific agents. Janson and co-workers [12] prepared a murine monoclonal antibody reactive with an idiotypic determinant of the hypervariable region of the TCR expressed by a patient's malignant leukemic cells. There was an 80% reduction in the number of leukemic cells after infusion of this monoclonal antibody. However, associated toxic side effects included fever, chills, nausea, vomiting, diarrhea, and shortness of breath. Monoclonal antibodies directed toward idiotypic determinants of the TCR may also be useful in the treatment of autoimmune disease because it has been observed that there is an extreme restriction in the TCR variable region usage in the T cells responsible for experimental allergic encephalomyelitis (EAE), a murine model of multiple sclerosis. The majority of T cells specific for myelin basic protein that can transfer the disease express a specific V[beta]8 phenotype [13]. Furthermore, in vivo treatment with a monoclonal antibody to V[beta]8 provided protection from disease transfer by encephalitogenic clonal T cells. Evidence for such restricted usage of the TCR variable region is being sought in spontaneous human neurological diseases, including multiple sclerosis and tropical spastic paraparesis.

Most anti-idiotypic antibodies utilized in treatment of human lymphoid malignancies are directed toward B cell immunoglobulin idiotypes. Idiotypic monoclonal antibodies to immunoglobulin were used [14] for treatment of patients with B cell lymphomas. The results were encouraging; one patient manifested an uninterrupted remission for more than 4 years after therapy. A partial response was observed in approximately half of the remaining patients. However, there were certain difficulties. During maturation, B cells use somatic hypermutation to increase their diversity. Thus, there is a tremendous idiotypic heterogeneity within the B cell population, which permits some neoplastic B cells to escape to attack of an antibody directed toward an individual idiotype. The TCR does not undergo somatic hypermutation and is less susceptible to this problem.

Monoclonal antibodies that recognize surface molecules that facilitate cell-cell interactions are also effective immunosuppressive agents. For example, antibodies specific for LFA-1 or its adhesion partner, ICAM-1, inhibit a series of T cell functions and thereby inhibit allograft rejection and GVHD [15]. The CD4 and CD8 proteins, which are important in T cell activation, recognition of major histocompatibility complex gene products,and cooperative cellular interactions, are also useful targets. In animal models, antibodies to CD4 or CD8 suppressed allograft rejection, GVHD, and autoimmune reactions [16]. Certain antibodies to CD4 are of special interest because they induce immunological tolerance in mice to simultaneously administered human, rat, and rabbit immunoglobulins. This form of tolerance was induced in T helper cells but not in B cells [17], and thus CD4 cells may be made tolerant when simultaneously confronted with certain antigens and monoclonal antibodies to CD4. Effective CD4 monoclonal antibody tolerance therapy is not confined to special protein antigens but is exploitable in transplantation. For example, short-term administration of a monoclonal antibody to CD4 induced cellular depletion and led to indefinite survival of adult pancreatic islet cell transplants in mice without subsequent immunotherapy [18]. Furthermore, administration of antibodies to CD4 ameliorated Type II collagen-induced arthritis in rats, murine EAE, and autoimmune myasthenia gravis [16].

The monoclonal antibodies to primate CD4 examined to date are not effective in tolerance induction in subhuman primate models. Despite this limitation, the administration of monoclonal antibodies to CD4 has led to improvement in preliminary clinical trials involving patients with severe rheumatoid or psoriatic arthritis [19].

The inducible [alpha] chain of the interleukin-2 receptor (IL-2R[alpha]) expressed on the surface of activated or abnormal T cells is also a target for effective monoclonal antibody immunotherapy [20]. Interleukin-2 receptor (IL-2R) is a useful target because resting cells do

not express the IL-2R[alpha], whereas the T cells participating in allograft rejection and abnormal T cells in certain autoimmune disorders express this receptor. To exploit this difference in expression, we used anti-Tac, a monoclonal antibody that blocks the binding of IL-2 to IL-2R[alpha], in the treatment of patients with human T cell leukemia/lymphoma virus-I (HTLV-I)-associated, IL-2R[alpha]-expressing adult T cell leukemia (ATL) [20]. The 20 patients treated in this study did not suffer any untoward reactions. Of the 20 treated patients, seven had remissions, three of these partial, one mixed, and three complete, lasting from 1 to more than 17 months after anti-Tac therapy. Unmodified anti-Tac has also been used for prevention of early allograft rejection episodes in patients receiving renal allografts. The IL-2/IL-2R system offers a variety of other possibilities for relatively specific immune intervention strategies that will be considered below.

Genetically Engineered Antibodies

Although murine antibodies are of value in therapy of human diseases, their effectiveness is limited because rodent monoclonal antibodies have a short survival time in humans and induce an immune response that neutralizes their therapeutic effect. Furthermore, the responses induced by murine antibodies are limited because they only weakly recruit human effector elements and are relatively ineffective as cytocidal agents. To circumvent these difficulties, genetically engineered antibody variants were produced that combine the rodent variable or hypervariable regions with the human constant or constant and variable framework regions [21-25]. The ability to genetically engineer antibodies represents a quantum leap in immune intervention that is comparable to the immunological revolution initiated by the introduction of monoclonal antibodies.

After the demonstration that lymphoid cells can express cloned transfected immunoglobulin genes, mouse-human chimeric monoclonal antibodies to tumors were generated with specificities directed toward antigens expressed by colorectal, mammary, pancreatic, and B cell and T cell malignancies [21, 22]. Jones et al. [23] proposed that because V domains represent a framework of [beta] sheets topped with antigen-binding loops and because [beta]-framework structures of most crystallized antibodies are nearly invariant, the specificity of the antibody combining site might be independent of the framework region. Thus, to further reduce the immunogenicity of rodent elements, Winter and colleagues generated humanized antibodies that retained only the antigen-binding complementarity-determining regions (CDRs) from the parent rodent monoclonal antibody in association with human framework regions [23, 24]. Unfortunately, in some cases humanized antibodies produced by this approach have reduced binding affinity for antigen when compared to the original rodent antibody. Queen and co-workers [25] addressed this problem in two ways. First, the human framework was chosen to be as homologous as possible to the original mouse antibody in order to reduce deformation of the transplanted mouse CDRs. Second, computer modeling was used to identify several amino acids in the mouse antibody framework that, although outside the CDRs, were likely to interact with the CDRs or antigen. These specific amino acids were retained in the humanized antibody.

One of the goals in the generation of humanized antibodies is the reduction of their immunogenicity. Although humanized antibodies are less immunogenic than their murine counterparts [26-28], the idiotypic element may be immunogenic. Furthermore, the presence of allotypes on the human immunoglobulin G (IgG) framework may provide foreign carrier determinants, thereby enhancing immune responses to the idiotypic element [29]. In the case of anti-Tac, the humanized version was dramatically less immunogenic than the parent murine monoclonal antibody when administered to cynomolgus monkeys [26]. Similarly, in the first clinical trial of a chimeric antibody, a murine human chimeric IgG1 antibody specific for a gastrointestinal tumor antigen elicited an antibody response against the chimeric antibody in only one of ten patients studied [27]. Furthermore, no anti-monoclonal antibody response was detected in two patients with non-Hodgkin's lymphoma studied who received CAMPATH-1, an antibody recognizing an antigen expressed by human lymphocytes [28]. The pharmacokinetics of an antibody molecule may also be altered genetically. The survival time of a monoclonal antibody can be altered to increase its period of effective action or, alternatively, to accelerate its clearance. For example, the pharmocokinetics of radiolabeled humanized anti-Tac differed substantially from that of murine anti-Tac when administered to

normal cynomolgus monkeys, with a prolongation of the survival half-time of humanized anti-Tac to 103 hours, as compared to 38 hours for marine anti-Tac [26]. Prolonged survival of humanized monoclonal antibodies in humans was observed by LoBuglio and co-workers [27]. The catabolic rate of an immunoglobulin is determined by the CH2 domain of the Fc region of the immunoglobulin. Thus, the longer survival of humanized antibodies observed probably reflects replacement of the murine IgG CH2 domain with the human IgG CH2 domain [30, 31].

Antibodies or their fragments can also be genetically engineered to have more rapid clearance. This might be desirable when a monoclonal antibody is conjugated to a radionuclide for use in radioimmunoscanning. For example, antigen-binding fragment (Fab), [F(ab').sub.2], or single chan Fv fragments of monoclonal antibodies have survival half-lives of less than 5 hours. Rapid turnover can also be accomplished by the deletion of the CH2 domain as demonstrated for an antibody reactive with the disaloganglioside GD2 expressed on human tumors of neuroectodermal origin [32].

Effector functions can be improved by introduction of human constant regions that impart biological activity to a murine antibody that lacks effector function but has the desired binding specificity. The human IgG subclasses differ in their antitumor activity and in their capacity to induce complement or antibody-dependent cell-mediated cytotoxicity. The IgGI subclass appears to be superior to the other subclasses in most functions [33]. The human IgGl versions of the CAMPATH-1 antibody and the L6 monoclonal antibody to a carcinoma-associated antigen were more effective in antibody-dependent cell-mediated cytotoxicity (ADCC) than the parent rodent antibodies [8, 24]. Furthermore, the humanized version of anti-Tac induces ADCC with human mononuclear cells, a function absent from the original mouse monoclonal antibody [34].

Human Monoclonal Antibodies

One solution to the problems of immunogenicity and poor recruitment of effector functions characteristic of rodent monoclonals is to produce human monoclonal antibodies. Human antibodies of appropriate specificity and of high affinity have been difficult to isolate. The use of mouse myeloma cells as fusion partners for human cells often leads to preferential loss of human chromosomes and instability of the hybrids. For ethical reasons one cannot immunize humans with certain tumor antigens. An alternative, the immortalization of human cells by Epstein-Barr virus, often generates lines that produce only low amounts of IgM-type antibodies. Human antibodies may be produced more easily with the use of SCID-hu mice [35], that is, immunodeficient mice reconstituted by human peripheral blood or human fetal thymus, bone marrow, and lymph nodes. When peripheral blood is used, antibodies may be produced if the donor has already been primed with antigen. Such antibodies have not yet been used in therapeutic trials.

An alternative approach to the production of human monoclonal antibodies has been reported that bypasses hybridoma technology [7, 36, 37]. The immunoglobulin V-region genes from B cells were cloned with the use of the polymerase chain reaction technique. The antibody derivatives were then expressed in E. coli and screened for ability to bind antigen. Initially, heavy chain V regions were expressed alone or with an irrelevant light chain V region. However, a large combinatorial library of the immunoglobulin repertoire of the mouse in phage lambda has now been generated [36]. Heavy and light chain libraries were prepared in phage lambda and used to generate a large array of random heavy plus light chain pairs expressed in bacteria in the form of Fab molecules. The screening for binding of antigens to hapten was rapid and permitted the analysis of many **monospecific Fab** -producing clones. In similar studies, Mullinax and co-workers [37] identified human antibody fragment clones specific for tetanus toxoid in a bacteriophage lambda immunoexpression combinatorial library prepared using messenger RNA derived from human peripheral blood lymphocytes. The bacteriophage clones are directly amenable to genetic manipulation for preparing complete immunoglobulins of the desired isotype. Further studies will be required to determine whether this approach will allow the efficient identification of human monoclonal antibodies of sufficiently high affinity for clinical studies.

Bifunctional Antibodies

Antibodies with two distinct binding activities have been generated to deliver radionuclides, toxins, cytotoxic drugs, or host cytotoxic cells to specific cellular targets [38]. Their predominant use has been to direct cytotoxic cells to target and lyse cells that they normally would not lyse.

Such bifunctional antibodies have been prepared by chemical cross-linking, disulfide exchange, or the production of hybrid-hybridomas (quadromas). Bispecific antibodies have also been produced by introduction of two sets of immunoglobulin heavy and light chains into myeloma cells or by construction of single-peptide bispecific antibodies with the use of peptide linkers between the variable domains of two distinct monoclonal antibodies. To be effective, the bispecific antibody must retarget the cytotoxic cell from its natural ligand to the ligand identified by the monoclonal antibody. Furthermore, the antibody must activate the cytotoxic cells into functional effectors without the normal major histocompatibility complex- and antigen-specific restrictions. The most effective bispecific monoclonal antibodies have the CD3 antigen on cytotoxic T cells or the CD16 Fc [gamma] R III receptor on natural killer cells as their nonantigen specificity. Bispecific antibodies with specificity against both tumor targets and CD3 or CD16 effector cells are effective in mediating the killing of tumor cells in vitro and in vivo. For example, murine anti-Tac does not participate in ADCC with human mononuclear cells. In contrast, murine anti-Tac-anti-CD3 and anti-Tac-anti-CD16 bifunctional agents used in conjunction with peripheral blood mononuclear cells killed targets that express IL-2 receptors. A universal bispecific antibody for retargeting effector cells to tumor cells can be generated with the use of a bispecific hybrid antibody with dual specificities for CD3 and for a rat immunoglobulin light chain allotype [39]. This bispecific antibody mediates retargeting of effector cells to a range of tumor cells, each coated with rat monoclonal antibodies bound to surface antigens.

Human T cells that had been retargeted by bifunctional antibodies were used to treat established human ovarian carcinoma in a nude mouse model [40]. Peripheral blood lymphocytes from patients were incubated overnight with IL-2 and treated with heteroconjugates containing antibody to CD3 cross-linked to an appropriate antibody to tumor and were injected interperitoneally into tumor-bearing mice. Tumor growth was inhibited. Bispecific antibodies have also been used in nontumor systems. For example, thrombolysis was enhanced by targeting of issue plasminogen activator (tPA) by bispecific antibodies to tPA and fibrin [4]. Thus, although problems associated with their manufacture remain to be resolved, bispecific antibodies show great promise as therapeutic agents.

Monoclonal Antibody-Cytotoxic Agent Conjugates

The limited efficacy of many unmodified monoclonal antibodies led to an alternative approach, the use of these agents as carriers of cytotoxic substances. An array of toxins of bacterial and plant origin have been coupled to monoclonal antibodies for production of immunotoxins [8, 41]. The strategy is to select from nature a toxic protein and then to modify the toxin so that it will no longer indiscriminately bind and kill normal cells but will instead kill only the cells expressing the antigen identified by the monoclonal antibody. The majority of toxins targeted to cell surfaces by immuno-conjugates act in the cytoplasm, where they inhibit protein synthesis. After binding to cell surface antigens, immunotoxins are taken up by endocytosis and delivered to endosomes. Fragments of some toxins (for example, diphtheria toxin) are then translocated across the membrane of this organelle. Other immunotoxins (for example, ricin) are routed further to the trans-Golgi network, where a minority undergo translocation to the cytoplasm. Unfortunately, most are routed to lysosomes, where they are degraded. In the cytoplasm, the toxins used clinically act either to adenosine diphosphate (ADP)-ribosylate elongation factor 2 (for example, Pseudomonas exotoxin) or to inactivate the 60S ribosomal subunit so that it has a decreased capacity to bind elongation factor 2 (for example, ricin). Less than ten toxin molecules in the cytoplasm are sufficient to kill the cell; however, more must bind to the cell surface to compensate for the inefficiencies in internalization and translocation.

Although immunotoxins are simple in concept, the first-generation immunotoxins were relatively ineffective. Several requirements must be fulfilled for an immunotoxin to be effective [41]. In particular, (i) the immunoconjugate should be specific and should not react with normal tissues. Binding to tissues that do not express antigen can be reduced by removal of the nonspecific natural cell-binding subunits or domains of the toxin. Furthermore, because plant glycoprotein toxins contain mannose oligosaccharides that bind to cells of the reticuloendothelial system and,

in some cases, also contain fucose residues that are recognized by the receptors on hepatocytes, deglycosylation of plant toxins may be required to avoid rapid clearance and potential cytotoxic effects on these cells. (ii) The linkage of the toxin to the antibody should not impair the capacity of the antibody to bind antigen. (iii) The immunotoxin must be internalized into endosomic vesicles. Thus, toxins directed by monoclonal antibodies to surface receptors that are normally internalized may be more active than those directed toward noninternalizing cell surface molecules. (iv) The active component of the toxin must translocate into the cytoplasm. These various goals can be in conflict; thus, the removal of the B chain of ricin reduces nonspecific binding but also reduces the capacity of the residual A-chain monoclonal antibody to translocate across the endosomic vesicle membrane. (v) For in vivo therapy, the linkage must be sufficiently stable to remain intact while the immunotoxin passes through the tissues of the patient to its cellular site of action. The first generation of heterobifunctional cross-linkers used to bind the toxin to the monoclonal antibody generated disulfide bonds that were unstable in vivo. This problem was solved in part by the synthesis of more stable cross-linkers, which used phenyl or methyl groups, or both, adjacent to the disulfide bond to restrict access to the bond.

The development by Pastan and co-workers [41] of IL-2R-directed Pseudomonas exotoxin (PE) conjugates for the treatment of IL-2R-expressing ATL demonstrates recent progress in the development of effective immunotoxins. PE, chemically coupled to anti-Tac, showed specificity in vitro [42]. However, only a few milligrams of this agent could be given to patients without producing liver damage because the toxin had not been sufficiently changed so that it would no longer bind to normal liver cells. Functional analysis of deletion mutants of the 66-kD PE [43] showed that Domain III was responsible for ADP-ribosylation of elongation factor 2; Domain II helped in translocation of the toxin to the cytosol, whereas Domain I was responsible for unwanted ubiquitous cell binding. A PE molecule from which the 26-kD Domain I had been deleted (PE40) had full ADP-ribosylating activity but extremely low cell-killing activity when used alone. PE40 conjugated to anti-Tac inhibited protein synthesis in T cell lines expressing Tac but not in lines not expressing the IL-2R. However, immunotoxins made by chemical attachment of this truncated PE to anti-Tac yielded a product that was heterogeneous. Active single-chain Fv fragments of antibodies have been produced in E. coli by linking of the light and heavy chain variable domains with a peptide linker [44]. Chaudhary and co-workers [45] used this genetic engineering approach to produce a single-chain antibody toxin fusion protein [anti-Tac (Fv)-PE40] in which the variable regions of anti-Tac were joined in peptide linkage to PE40 to generate an immunotoxin that was cytotoxic to human cell lines bearing IL-2R and to freshly obtained ATL cells but not to receptor-negative cells.

The antitumor activity of immunotoxins has been evaluated in animal models since their introduction [46]. Treatment usually delayed the appearance of tumors and prolonged the lifespan of the animals. In a few cases, there was complete regression of the tumor.

Several in vivo clinical trials in humans have involved the parenteral administration of immunotoxins. In certain cases, for example, with monoclonal antibody-toxin conjugates directed toward breast or ovarian cancers, severe neurological toxicity was observed because of an unanticipated crossreactivity of the monoclonal antibody with an antigen in the central nervous system [47]. The most common toxicity observed was capillary leakage that resulted in hypoalbuminemia, edema, fatigue, and myalgia.

The results of in vivo clinical trials in patients with cancer with first-generation immunotoxins did not fulfill the hopes engendered by in vitro and animal model studies. There were only two complete remissions and eight partial remissions among the 127 patients treated in ten clinical trials with toxin-conjugated monoclonal antibodies directed toward ovarian, breast, colorectal, and lymphoid neoplastic cells. More encouraging results were obtained when benign diseases were treated with immunotoxins or when modified immunotoxins were used in therapy of patients with cancer. For example, 9 of 22 patients manifested a mixed or partial response, and one had a complete remission after treatment of a refractory B cell leukemia-lymphoma with a monoclonal antibody to CD19 conjugated to a modified ricin toxin that had galactose binding sites blocked sterically [48]. Furthermore, 22 of 32 evaluable patients with GVHD had a favorable

early response in at least one organ after therapy with monoclonal antibody to CD5 conjugated to ricin A [49].

An alternative approach for the delivery of cytotoxic agents to cancer cells involves the use of monoclonal antibodies as carriers for enzymes to tumor cell surfacs [50]. The enzymes are chosen for their ability to convert drug precursors injected parenterally into active antineoplastic drugs. The active cytotoxic agents formed can then penetrate nearby tumor cells and cause the death of these cells. A number of prodrugs (drugs in an inactive form that can be transformed at the tumor into active anticancer drugs by antibody-enzyme conjugates) have been developed. The antibody-enzymes conjugates were shown to localize to tumors through the activity of the monoclonal antibodies that bind to tumor-associated antigens. In vivo studies showed that prodrug administration after monoclonal antibody-enzyme infusion can result in antitumor activities significantly greater than the activities of the prodrugs, drugs, or monoclonal antibodies given alone.

Radiolabeled Monoclonal Antibodies

Toxin conjugates do not pass easily from the endosome to the cytosol. Furthermore, the toxins are immunogenic and thus provide only a short therapeutic window before the development of antibodies directed toward the toxin. Radiolabeled monoclonal antibodies have been developed as alternative immunoconjugates for delivery of a cytotoxic effector to target cells and for radioimaging. [8,15] Radioummunodetection with the use of radiolabeled monoclonal antibodies, most often with monoclonal antibodies to carcinoembryonic antigen, is widely used to complement other approaches for tumor detection. Although intact IgG antibodies are retained better by tumors and thus appear to be better for therapy, [F(ab').sub.2] and Fab fragments are preferred for imaging because both targeting and blood clearance are more rapid, which reduces the background. Tumors as small as 0.5 cm, which are sometimes missed by other radiological methods, can be imaged with antibodies or antibody fragments labeled with suitable radionuclids.

One advantage in the use of radiolabeled monoclonal antibody conjugates for therapy is that with the appropriate choice of radionuclide, radiolabeled monoclonal antibodies can kill cells from a distance of several cell diameters and may therefore kill antigen-negative cells adjacent to antigen-expressing cells. Furthermore, the radiolabeled antibody need not be internalized to kill the tumor cell.

In a radiolabeled monoclonal antibody, the radionuclide must be tightly linked to the antibody either directly or by a bifunctional chelate. For a monoclonal antibody-chelate complex to be effective, it must meet criteria in addition to those that are true for all monoclonal antibodies: (i) the chelating agent coupled to the monoclonal antibody should not compromise antibody specificity; (ii) the chelation and radiolabeling procedure should not alter the distribution and catabolism of the monoclonal antibody; and (iii) the bifunctional chelate should not permit elution and thus premature release of the radiolabeled metal in vivo. Failure to fulfill this last requirement has led to unacceptable toxicity and reduced efficacy. There are a number of suitable [alpha]-, [beta]-, and [gamma]-emitting radionuclides. Isotopes emitting [beta] particles, although superior to [gamma]-emitting radionuclides, are not optimal because their low linear energy transfer released over a relatively long distance results in inefficient local killing of target cells coupled with toxicity to distant normal tissues. Nevertheless, [beta]-emitting radionuclides such as [sup.131.I], [sup.90.Y], [sup.186.Re] and [sup.67.Cu] have been useful in immunotherapy. For example, hepatoma-bearing patients have been successfully treated with [sup.131.I]-labeled antibodies to ferritin [52]. Furthermore, [sup.90.Y]-labeled antibodies to ferritin combined with autologous marrow transplantation resulted in complete remissions in four of eight patients with Hodgkin's disease [52]. [sup.90.Y]-labeled anti-Tac was effective in prolonging the survival of cardiac allografts and xenografts in a subhuman primate model [53]. In a subsequent trial, [sup.90.Y]-labeled anti-Tac was evaluated for the treatment of patients with HTLV-I-associated, Tac-expressing ATL. At the doses used (5 and 10 mCi per patient), no toxicity was observed in five of six patients studied; modest granulocytopenia and thrombocytopenia were observed in one patient. Five of these six patients underwent a sustained partial or complete remission after [sup.90.Y]-labeled anti-Tac therapy.

Future development of isotopic monoclonal antibody chelates may focus

on [alpha]-emitting radionuclides, which may be the most effective at killing tumor targets without damaging distant normal tissues [51,54]. Radionuclides emitting [alpha] particles release high energy emissions (6 to 9 MeV, ten times as great as [beta]- or [gamma]-emitters) over a short distance (40 to 80 [micrometer]) and are efficient at killing individual target cells, such as those found in leukemia, without significantly penetrating normal tissues. Under hypoxic conditions that permit little cellular repair, [alpha] irradiation is efficient at killing cells. Suitable [alpha]-emitting nuclides available for immunotheraphy include [sup.211.At], [sup.212.Pb], and [sup.212.Bi]. [sup.212.Bi] chelated to anti-Tac was used with a limiting dilution colong-forming assay to demonstrate that [sup.212.Bi] conjugated to anti-Tac was well suited for immunotherapy [54]. Activity levels of 0.5 [mu]Ci, or the equivalent of 12 rad/ml of [alpha] irradiation, targeted by [sup.212.Bi]-labeled anti-Tac eliminated greater than 98% of the proliferative capacity of IL-2R-expressing cells with only a modest effect on IL-2R-negative lines. Thus, one of the most promising directions for future development of armed monoclonal antibodies for the treatment of cancer involves the chelation of [alpha]-emitting radionuclides to human or humanized monoclonal antibodies. Such conjugates may prove to be relatively nonimmunogenic agents that are effective in eliminating malignant cells when used alone or as part of multimodality treatment with conventional chemotherapy/

Since the development of monoclonal antibody technology, the medical community has applied these agents to in vivo diagnosis and therapy of human disease. Recent advances in linkage of toxins and isotopes to monoclonal antibodies and in genetic engineering of antibodies has led to reduced immunogenecity and has improved effector function, thus providing new potential for the prevention of allograft rejection and for the treatment of neoplastic, infectious, and autoimmune diseases. We have come close to fulfilling the vision of Ehrlich who, in ending his Croonian lecture, On Immunity with Special Reference to Cell Life in 1900, stated, "It is to be hoped that such immunisations as these, which are of great theoretical interest, may also come to be available for therapeutic application. The idea has already been mooted by v. Dungern, of attacking epithelial new formations, particularly carcinoma, by means of specific 'antiepithelial sera.' . . . I trust, my lords and gentlemen, that from what I have said you may have obtained the impression, to allude again to my quotation from Bacon, that we no longer find ourselves lost on a boundless sea but that we have already caught a distinct glimpse of the land which we hope, nay, which we expect, will yield rich treasures for biology and therapeutics." [5]

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